Immunotherapy using PD-1/PDL-1 inhibitors in metastatic triple-negative breast cancer: A systematic review

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Abstract

Breast cancer is the most commonly diagnosed cancer in women and is one of the leading causes of death from cancer in women worldwide. Despite the significant benefits of using conventional chemotherapy in the treatment of breast cancer, one of its subtypes, the triple-negative breast cancer, is still a challenge in clinical practice. Recent studies have been investigating the role of the immune system in breast cancer and the development of immunotherapy. Although recently the use of atezolizumab, an anti-PD-L1 monoclonal antibody, combined with chemotherapy was approved, an important step in the treatment of patients with triple-negative metastatic breast cancer, the use of immunotherapy to treat breast tumors remains a major challenge. In this systematic literature review, following PRISMA guidelines, we searched for clinical trials using immunotherapy in the treatment of metastatic triple-negative breast cancer published until March 2020 in the databases EMBASE, PubMed and Cochrane Central Register of Controlled Trials (CENTRAL), with no language restrictions. We did not contact the authors of the clinical trials to obtain additional information. Two researchers independently collected the data and assessed the quality of this study. The literature shows that immunotherapy with anti-PD-1/PD-L1 agents is emerging as a new treatment option in breast cancer. On the other hand, when compared to other types of cancer in which several agents have already been approved, the research is still in its infancy. The use of anti-PD-1/PD-L1 agents as monotherapy revealed encouraging results in the metastatic setting, especially when administered in the early course of the disease, although combination strategies with chemotherapy appear to increase its efficacy. The main limitation of this study is the approach of cancer only in advanced stages.

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Key words: Triple-negative breast cancer; immunotherapy; PD-1; PD-L1; checkpoint inhibitors.

Contributions: DFT, VCR and RLB contributed to conceptualization, investigation, writing-original draft, writing-review, visualization, manuscript critical revision and editing. MAVA, LMCJ, GRV and TRGT contributed to investigation, writing-original draft, writing-review. All authors have read and approved the manuscript.

Funding: this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Conflicts of interests: All authors declare no conflict of interests.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author, D. F. T., upon reasonable request.

Ethics approval and consent to participate: this article does not contain any studies with human participants or animals performed by any of the authors.

Received for publication: 27 May 2020.
Revision received: 8 October 2020.
Accepted for publication: 9 October 2020

Oncology Reviews 2021; 15:497
doi:10.4081/oncol.2021.497

Introduction

Breast cancer is a disease that manifests itself in different ways in each woman and can be classified based on the expression or lack of expression of protein receptors, such as estrogen receptor (ER), progesterone receptor (PR) and receptor for human epidermal growth 2 (HER-2).[1] The term *triple-negative* refers to the fact that this type of tumor does not express any of the three most used biomarkers in the classification of breast cancer: ER, PR and HER-2 protein. Only since 2005 it was possible to identify this subtype, and despite extensive research and initiatives on the subject, little is known about the origin of this type of tumor.[1]

In 2018, breast cancer was responsible for more than 2 million new cancer cases and 626,679 deaths worldwide, being the most commonly diagnosed cancer in women and one of the leading causes of death from cancer in women worldwide. It is epidemiologically estimated that triple-negative breast cancer (TNBC) is responsible for approximately 15 to 20% of all breast carcinomas, being associated with younger age, aggressive clinical course and worse prognosis when compared to other histological types of cancer.[2-5]

In the last few years, several studies regarding the immune

system in breast cancer have been carried out, mainly to understand the immune response role in TNBC. An example of its importance is that the presence of tumor infiltrating lymphocytes (TILs), assessed by immunohistochemical staining, is widely recognized as a predictor of good prognosis in both contexts of adjuvant and neoadjuvant treatment of TNBC.[6-8]

In addition to the presence of TILs, the expression of immune evasion molecules in the tumor microenvironment, such as programmed death ligand 1 (PD-L1), can affect the prognosis of TNBC. PD-L1 binds to the programmed death receptor 1 (PD-1), a checkpoint protein in immune cells. This binding acts as a type of *off switch* that prevents T cells from attacking other cells in the human body. This mechanism influence the prognosis of TNBC, as shown in some previous studies evaluating the effect of PD-L1 inhibitors in the treatment of TNBC.[9-13]

The programmed death receptor 1 (PD-1) pathway plays a critical role in regulating the immune response. PD-1, an immune checkpoint inhibitor receptor expressed on activated T cells, B cells, natural killer cells, activated monocytes, dendritic cells, myeloid cells and a subset of thymocytes, limits autoimmunity by regulating the activity of effector T cells in the periphery in response to an inflammatory stimulus. PD-L1, a PD-1 ligand, acts as an immunosuppressive signal and is up-regulated in response to pro-inflammatory signals, such as interferon-γ.[10-13]

Despite showing durable clinical benefits in several tumor types, immunotherapy with PD-1/PD-L1 inhibitors is associated with adverse effects related to its toxicity.[14-16] New studies have been conducted in order to develop predictive biomarkers for therapy with PD-1 or PD-L1 agents, aiming to minimize the exposure of patients with low probability to respond to this treatment. Some strategies, such as tumor-cell PD-L1 expression, microsatellite instability and loss of DNA repair enzymes, are already being used in clinical practice, although more studies are needed to prove its accuracy and predictive values.[17] Other strategies, like mutational burden and tumor-infiltrating lymphocytes, also have been showing promising results as good therapeutic response predictors.[18,19]

Immunotherapy has been used as monotherapy and mainly in a combination strategy with chemotherapy. The main point is how to choose the best agent to use in combination regimens. Most of combination regimens use drugs that were shown to be possibly related to enhanced breast cancer immunogenicity. The classical examples are the anthracyclines, platinum salts and taxanes.[20] Chemotherapy by itself can induce multiple immunomodulatory alterations in the tumor microenvironment and those alterations can positively influence immunotherapy efficiency.[21,22]

Taking into account the lack of effective treatments in this subtype of breast cancer, together with the development of new therapeutic agents that are directed against immune checkpoint molecules, such as anti-PD-1 and anti-PD-L1 monoclonal antibodies, we have support for the assessment of the state of the art of clinical research with immunotherapeutic approaches through PD-1/PD-L1 inhibitors in the treatment of metastatic TNBC.

Methodology

Strategy of search and selection of studies

This systematic review followed PRISMA Guidelines for systematic reviews.[23] A search for references was carried out in EMBASE, PubMed and Cochrane Central Register of Controlled Trials (CENTRAL), until March 2020, without language restrictions. The EMBASE, PubMed and Cochrane Library databases were systematically searched from creation dates until March 31, 2020. The key words and Medical Subject Headings (MeSH) were *'breast cancer', 'triple-negative breast cancer'* or '*TNBC'* in association with '*Immunology', 'Immunotherapy'* and '*Immune checkpoint inhibition',* and '*clinical trial'* or '*clinical trials'* associated with '*PD-1 inhibitor'* or '*PD-L1 inhibitor*'. We identified the original randomized controlled trials and did not obtain any additional information by contacting the authors of the primary studies. The bibliographic references of all selected articles were analyzed in order to find possible clinical trials to be included.

The screening in the databases was performed by two of the researchers, identified by the initials DFT and LMCJ, to find relevant studies based on their titles. When one or both of the evaluators disagreed on the fulfillment of the inclusion criteria for a given study, the disagreements were resolved by consensus without the need for assistance from a third researcher.

Eligibility criteria

The studies were included if they met the following criteria: i) the participants, aged over 18 years, were diagnosed with advanced/metastatic triple-negative breast cancer confirmed by immunohistochemistry; ii) randomized clinical trials comparing the intervention with immunotherapy to another group without immunotherapy; iii) trials with immunotherapy regardless of the drug, dosage and route of administration, inasmuch as their action was to inhibit the PD-1/PD-L1 system; and iv) the trials provided relevant and complete data, from selection to research outcomes.

The studies were excluded if they met the following criteria: i) literature reviews; ii) animal studies; iii) there were contraindications for immunotherapy related at any time point; iv) the articles were not available or the data had not been published; or v) immunotherapy studies with targets unrelated to PD-1/PD-L1 inhibition or immunotherapy with anti-PD-1/PD-L1 agents in association with other immunotherapeutic agents.

Extraction of data

The literature search was conducted with the use of the parameters described in eligibility criteria, in the previously mentioned databases, with the assistance of five research strategies, varying MeSH/DeCs descriptors and Boolean operators. Following the recommendations of the *Cochrane Effective Practice and Organisation of Care,*[24] the articles compiled in the initial stage were analyzed. Data collection was performed on an electronic form designed for this purpose, containing the following variables: authors of the study and publication year, country of study, study design, type of drug used, number of patients studied and results.

Evaluation of methodological quality

The studies were analyzed and classified according to the levels of evidence from *Grading of Recommendations Assessment, Development and Evaluations* (GRADE).[25] Reliability between examiners was measured with the kappa function.[26] The calculations resulted in a Kappa value of 0.9, that is considered excellent.

Results

From the published studies, 568 potentially eligible clinical trials were selected by their titles and abstracts. After screening, 29 records were completely read. A manual search of the reference lists in those studies did not reveal any additional eligible studies. Eventually, 08 records were in compliance with the inclusion criteria and were included in this review (shown in Table 1). The

screening process of this study is illustrated in Figure 1.

The main demographic and clinical characteristics were extracted from the articles and are shown in Table 1, representing the group of patients with TNBC. The KEYNOTE-150 study[27] and the KEYNOTE-086 Cohort B[28,29] have not published all their data yet, therefore they were not included in Table 1.

The therapeutic interventions, quality of evidence, mechanisms of action and the final results are described in Table 2. The number of patients in the studies refers only to the individuals who received the drug treatment described and had a confirmed diagnosis of TNBC. PD-L1+ expression was considered when detected in in stroma or in ≥1% of tumor cells by immunohistochemistry (IHC), as defined by a combined positive score (CPS) - a ratio between PD-L1-positive cells (tumor or immune cells) and the total number of tumor cells ×100 - being ≥1. To date, IHC is the only Food and Drug Administration (FDA)-approved test for measuring PD-L1 expression.

The adverse effects reported in 2 or more individuals participating in the study were included and are described and shown in Table 3. The adverse effects classified by the authors with a grade of 3-5 or immune-mediated were included if they appear in 1 or more individuals and are described in Table 3. The KEYNOTE-150 study[27] and the KEYNOTE-086 Cohort B[28,29] have not published all their data yet, therefore they were not included in Table 3. The adverse effects of the JAVELIN study[30] are reported together in the published article, making it impossible to distinguish those referring to TNBC, so they were not included in this table.

Discussion

Pembrolizumab

Pembrolizumab (MK-3475) is a monoclonal antibody that targets the programmed death receptor 1, a transmembrane protein in T cells. Pembrolizumab is a humanized monoclonal antibody that binds to PD-1. The binding of the antibody to PD-1 prevents the interaction between programmed death-1 in T cells and the ligand PD-L1 in tumor cells and therefore the immune response is not prevented and is increased to eliminate abnormal effects in tumor cells.[31-34] The KEYNOTE-012[31] study was a non-randomized, 1b phase, multi-cohort study that included a subset of patients with metastatic TNBC. Tumors were screened for PD-L1 activity of at least 1% expression in tumor cells or stroma, using the anti-human 22C3 antibody PD-1 (Merck & Co., Kenilworth, NJ, USA) and was identified in almost 60% of the examined patients. Of the 111 patients with TNBC, 58.6% had positive PD-L1 tumors.[31]

Thirty-two women were recruited to assess the antitumor activity and safety of pembrolizumab. The dose of pembrolizumab was 10 mg/kg in every 2 weeks. There was no restriction on the previous lines of treatment for inclusion. Pembrolizumab was administered at a dose of 10 mg/kg until progression or toxicity. The primary endpoint of the study was the objective response rate (ORR). The patients were strongly pretreated, with a mean number of two pretreatment lines (ranging from 0 to 9), 100% of patients were already exposed to taxanes, 71% to anthracyclines and 65% to capecitabine. Most patients (78%) had visceral involvement. Among the 27 patients evaluated for tumor response, the ORR was 18.5%, including 1 complete response (CR) and 4 partial responses

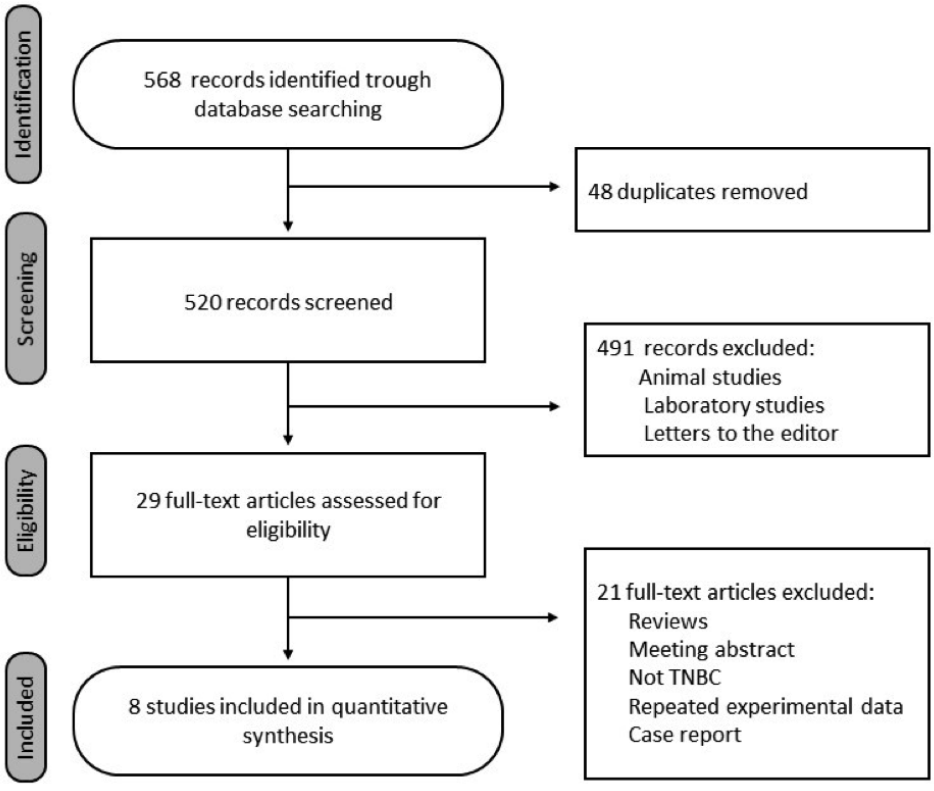

Figure 1. The PRISMA flow diagram.

Table 1. Baseline patients demographics and clinical characteristics.

Demographics and clinical characteristics	Nanda *et al.*, 2016[31]	Loi *et al.*, 2017*[28] Adams *et al.*, 2019*[29] Results of cohort A	Dirix *et al.*, 2018[30]	Emens *et al.*, 2019[39]	Schmid *et al.*, 2018*[40] Emens *et al.*, 2019*[41]
	Value	Value	Value	Value	Value
Age, years, median (range)	50.5 (29-72)	53.5 (28–85)	52.5 (31-80)	53 (29-82)	55 (20-82)
Female, No. (%)	32 (100)	170 (100)	58 (100)	--	448 (99.3)
Race, No. (%)					
White	25 (78.1)	--	45 (7.8)	--	308 (68.3)
Black or African American	7 (21.9)	--	9 (15.5)	--	26 (5.8)
Asian	--	--	1 (1.7)	--	85 (18.8)
Other	--	--	3 (5.2)	--	--
ECOG performance status, No. (%)					
0	14 (43.8)	90 (52.9)	33 (56.9)	53 (46)	256 (56.9)
1	18 (56.3)	80 (47.1)	25 (43.1)	61 (53)	193 (42.9)
2	--	--	--	--	1 (0.2)
Smoking history, No. (%)					
Never smoker	--	--	36 (62.1)	--	--
Current or former smoker	--	--	17 (29.3)	--	--
Unknown		--	--	5 (8.6)	-- --
Location of metastases, No. (%)					
Brain	3 (9.4)	--	--	--	30 (6.7)
Bone	--	--	--	34 (29)	145 (32.2)
Liver	--	--	--	--	126 (27.9)
Lung	--	--	--	--	226 (50.1)
Lymph node only	--	--	--	--	33 (7.3)
Visceral	25 (78.1)	--	--	75 (65)	--
Non-visceral	7 (21.9)	--	--	--	--
LDH level, No. (%)					
<1 ULN	--	82 (48.2)	--	--	--
>ULN	13 (40.6)	--	--	--	--
>2×ULN	5 (15.6)	--	--	--	--
≥2.5 ULN	--	2 (1.2)	--	--	--
No. of prior therapies for metastatic disease					
Median	(range)	2 (0-9)	--	2 (1-6)	-- --
0, No.	(%)	5 (15.6)	--	--	21 (18) --
1, No.	(%)	6 (18.8)	53 (31.2)	--	28 (24) --
2, No.	(%)	6 (18.8)	43 (25.3)	16 (27.6)	-- --
3, No.	(%)	5 (15.6)	31 (18.2)	--	-- --
4, No.	(%)	2 (6.3)	22 (12.9)	--	-- --
≥5, No.	(%)	8 (25.0)	21 (12.4)	--	-- --
Previous neoadjuvant or adjuvant therapy, No. (%)	28 (87.5)	141 (82.9)	--	--	284 (63.0)
Previous chemotherapy exposure, No. (%)					
Taxane	32 (100.0)	--	--	109 (94)	231 (51.2)
Anthracycline	23 (71.9)	--	--	99 (85)	243 (53.9)
Capecitabine	21 (65.6)	--	--	--	--
Bevacizumab	--	--	--	24 (21)	--
Platinum	19 (59.4)	--	--	67 (58)	--
Eribulin	8 (25.0)	--	--	--	--

*Studies from the same group of researchers at different stages of study; (--) Information not available in the published article. ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; ULN, upper limit of normal.

(PR). The average duration of the response was not achieved, but ranged from 15 weeks to >47 weeks. The 2-years survival rate was 22%. Notably, the baseline LDH level was associated with rapid tumor progression. Five patients (15.6%) developed grade 3 or higher toxicity, including one treatment-related death due to disseminated intravascular coagulation.[31]

Adverse events related to immunity were described, such as colitis, hepatitis and hypothyroidism, or side effects such as nausea, myalgia, fatigue and arthralgia were also importantly reported according to Table 3 of the results. Antitumor activity was assessed in 27 of 32 women who received pembrolizumab and the overall response rates were 18.5%. The complete response was observed in 1 patient (3.7%), the partial response was observed in 4 (14.8%) and 7 (25.9%) presented stable disease. These results provide evidence that pembrolizumab, administered in every 2 weeks, to patients who had metastatic TNBC previously treated with chemotherapy had clinical activity and an acceptable safety profile.[31]

The KEYNOTE-086[28,29] study is a phase II clinical trial, divided into two parts, currently still active, for patients with metastatic TNBC receiving pembrolizumab. Part 1 examines the safety and efficacy of pembrolizumab and included 2 cohorts, cohort A, that had patients with metastatic TNBC who received at least 1 previous systemic treatment for metastatic disease with documented disease progression in the most recent therapy, and cohort B, with patients with positive PD-L1 metastatic TNBC who did not receive previous systemic treatment for metastatic disease.[28,29]

The second part of the trial is an expansion of cohort A with tumors strongly positive for the expression of PD-L1 and will be started only if there is ≥1 response. Patients will receive Pembrolizumab 200 mg IV in every 3 weeks until disease progression, intolerable toxicity or patient or investigator decision.[28,29]

The objective response rate is the primary outcome and was 4.7%. The CR was observed in 0.6% and the PR was observed in 4.1%. 20.6% of the patients had stable disease (SD).[28] Response duration, disease control rate, progression-free survival (PFS) and overall survival (OS) were the secondary end points of the study. The disease control rate was 7.6%, the median PFS was 2 months and the median OS was 8.9 months.[29] PFS and OS were not significantly changed based on PD-L1 status. Sixty percent of patients had treatment-related adverse events, with fatigue and nausea being the most common adverse events. Immune-mediated adverse events, including hypothyroidism, hyperthyroidism and pneumonitis have also been reported.[31,32]

Pembrolizumab has also been studied in association with eribulin in a multicenter, single-arm, open (I/II) (KEYNOTE-150) study with the aim of examining the safety and activity of the combination in patients with metastatic TNBC.[27] Eribulin is a chemotherapeutic drug, with anti-microtubule action registered in previously treated metastatic BC. The patients in this study could have been previously treated with 0 to 2 lines of chemotherapy for metastatic disease. A total of 107 patients, 106 of whom were evaluated, were included, regardless of PD-L1 status. The ORR was 26.4% (3 patients with CR and 25 with PR) and CBR was 32.8%.[27] It is important to note that the ORRs were not significantly different concerning the PD-L1 status (30% in positive PD-L1 *vs*. 22% in negative PD-L1; of the three patients who had a complete response, one patient was PD-L1 negative) or previous exposure to chemotherapy (29% in untreated patients *vs* 22% in patients with 1-2 previous lines).[27] The combination of immunotherapy with chemotherapeutic agents such as eribulin, can induce immunomodulatory changes in the tumor, such as the positive regulation of PD-L1 and hyperexpression of immunogenic markers on the cell surface, where these changes in the tumor environment together can positively influence the effectiveness of immunotherapy.[21,22]

The results mentioned above are favorably correlated with those obtained with the single agent eribulin.[35] The responses duration was long (median of 8.3 months, lasting more than 6 months in 53% of responders), and the median of PFS and OS was 4.2 and 17.7 months, respectively. The adverse effects related to the combination treatment were comparable to those seen in each treatment as monotherapy. The most common adverse events were asthenia, nausea, peripheral sensory neuropathy and alopecia. Thus, a combination of eribulin and Pembrolizumab was well tolerated and demonstrated antitumor activity in patients with metastatic TNBC.[27]

Avelumab

Avelumab (MSB0010718C) is a monoclonal antibody that targets the programmed cell death ligand 1 receptor, a transmembrane protein in tumor cells. Avelumab is a fully human IgG1 monoclonal antibody that binds to PD-L1.[30] Avelumab was previously the first approved drug for metastatic by the FDA in metastatic merkel cell and advanced urothelial carcinoma.[36] Since its approval, new studies changing the dosage based on body weight to a flat dose of Avelumab in Metastatic Merkel Cell and Advanced Urothelial Carcinoma and its applicability in other types of tumors have been studied.[30,37]

The JAVELIN study, a phase Ib study, evaluated the action of avelumab on solid tumors through a cohort of 168 patients, in which 58 patients had metastatic breast cancer. The dose of Avelumab was 10 mg/kg IV in every 2 weeks until progression. To be eligible, patients had to have a biopsy-proven locally advanced or metastatic breast cancer, have received ≤3 previous lines of chemotherapy and have previously received anthracycline and taxane, unless contraindicated.[30]

The ORR in those patients with metastatic breast cancer was 3.0%, including 1 CR, 4 PRs and 42 patients with SD. Of the 5 patients who had a response, 3 had TNBC. In the cohort of patients with metastatic breast cancer, 58 (34.5%) patients had TNBC. The ORR in the TNBC cohort was 5.2%, with 0 patients with complete response, 3 patients with partial response and 15 with stable disease. PD-L1 expression of at least >1% was seen in 48 of 58 patients with TNBC. The ORR for those with TNBC based on PD-L1 status was 6.1% [PDL1≥1% (n=33)], 7.7% [PDL1≥5% (n=13)] and 0% [PDL1≥25% (n=2)]. In tumor-associated immune cells with PDL1≥10%, the ORR was 22.2%.[30]

One hundred and fifteen patients in the general study showed adverse events, with fatigue, infusion-related reactions and nausea being the most common adverse events. As well as in other studies, adverse effects related to immunity were reported including hypothyroidism, autoimmune hepatitis, pneumonitis, thrombocytopenia, among others that can be better observed in Table 2. The results of this study show that the safety profile of Avelumab is tolerable and that those who have TNBC with PDL1-positive immunohistochemistry appear to have a clinical benefit with avelumab.[30]

Atezolizumab

Atezolizumab (MPDL3280A), such as avelumab, is a monoclonal antibody that targets the programmed cell death ligand 1 receptor, a transmembrane protein in tumor cells. Unlike Avelumab, Atezolizumab is a humanized monoclonal antibody of the IgG1 isotype that selectively binds to PD-L1.[27,28]

The first study to assess the safety of Atezolizumab in patients with advanced solid tumors or locally metastatic tumors was established from a phase I, open, dose-escalation study

(NCT01375842). Sixty-nine percent of the cohort assessed for safety had PD-L1 expression of at least ≥5%, and all patients assessed for efficacy had PD-L1 expression of at least ≥5%. The ORR was 19% (2 CRs, 2 PRs and 3 SDs).[38]

In that phase I clinical trial, atezolizumab led to a higher ORR in the first-line scenario (24%) compared to a second-line or higher scenario (6%). In first-line patients, the median OS was 17.6 months. Interestingly, patients with PD-L1 expression in at least 1% of immune cells infiltrated in tumors had higher ORRs (12 *vs* 0%) and longer OS (10.1 *vs* 6.0 months) than those with PD-L1 expression in less than 1% of immune cells infiltrated in tumors. High levels of immune cells (>10%) were independently associated with higher ORR and higher OS. There were 3 patients who had pseudoprogression, but eventually had tumor retraction. Adverse drug-related events occurred in 63% of patients and grade 3 toxicity occurred in 11%. One patient had grade 4 pneumonitis. The most common drug-related adverse events were fatigue, fever and nausea.[39]

In the IMpassion130 study, the study that showed more benefits in the use of immunotherapy to treat TNBC, a PD-L1 expression above 1% in immune cells was used to define the PD-L1+ group.[40] Interestingly, most of the patients that tested positive for PD-L1+ in tumor-infiltrating immune cells also had a positive expression of PD-L1 in tumor cells. In the IMpassion130[41] biomarker subgroup analysis, the expression of PD-L1 in immune cells was positively correlated with the number of CD8 + T cells, and both factors were associated together with the increase in PFS and OS.[34] The association with nab-paclitaxel was chosen a priori in the IMpassion130 study because it facilitates the reduced use of corticosteroids.[42,43] Although reducing the use of corticosteroids in oncology is very relevant, other studies have shown that better agents may be available to increase the immunogenicity of breast cancer, citing anthracyclines, platinum salts and other taxanes.[20]

In the phase III randomized study IMpassion-130,[40,41] patients with metastatic TNBC treated with first-line and with good performance status (0-1) were randomized to weekly receive nab-paclitaxel (100 mg/m² D1, D8, D15) plus atezolizumab (800 mg D1, D15) or placebo, in every 28-day cycle.[40] Previous treatments such as radiotherapy and chemotherapy, including taxanes, were allowed if they were performed at least 12 months before randomization. Patients with treated asymptomatic CNS metastases were eligible. Patients were stratified according to the presence of liver metastases, previous taxanes in the adjuvant and/or neoadjuvant setting and the PD-L1 expression in the immune cells infiltrated in the tumor (<1% *vs* at least 1%) by IHC using the Ventana assay described above.[40,41]

The primary endpoint was PFS, with a subsequent change to include the OS as a co-primary. Both parameters had to be tested

Table 2. Characteristics of the included studies and details of the interventions.

Trial	Reference	Quality of evidence	Setting	Therapy	Drug	Action	Patients	Results
KEYNOTE-012 NCT01848834	Nanda *et al.*, 2016[31]	High	Advanced PD-L1+ TNBC	Single agent immunotherapy	Pembrolizumab 10 mg/kg Q2W	PD-1 inhibitor	32	ORR, 18.5% Median PFS, 1.9 months Median OS, 11.2 months
KEYNOTE-086 NCT02447003	Loi *et al.*, 2017*[28] Adams *et al.*, 2019*[29]	High	Advanced, untreated, any PD-L1 TNBC (cohort A) Advanced, untreated PD-L1+ TNBC (cohort B)	Single agent immunotherapy	Pembrolizumab 200 mg Q3W	PD-1 inhibitor	Cohort A: 170 Cohort B: 84	ORR Cohort A, 4.7% ORR Cohort B, 22.6% Median PFS Cohort A, 2 months Median PFS Cohort B, 2.1 months Median OS Cohort A, 8.9 months Median OS Cohort B, 19.2 months
KEYNOTE-150 NCT02513472	Tolaney *et al.*, 2018[27]	High	Advanced TNBC unselected for PD-L1	Combination of immunotherapy and chemotherapy	Eribulin ± pembrolizumab 200 mg Q3W	PD-1 inhibitor + Chemotherapy	107	ORR, 26.4% (30.6% in PD-L1+) Median PFS, 4.2 months Median OS, 17.7 months
JAVELIN NCT01772004	Dirix *et al.*, 2018[30]	High	TNBC unselected for PD-L1 (68.8% had PD-L1+ tumors)	Single agent immunotherapy	Avelumab 10 mg/kg every 2 weeks	PD-L1 inhibitor	58	ORR, 5.2% (22.2% in PD-L1+) Median PFS, 1.5 months Median OS, 9.2 months
NCT01375842	Emens *et al.*, 2019[39]	High	Advanced TNBC unselected for PD-L1 (65.7% had PD-L1+ tumors)	Single agent immunotherapy	Atezolizumab 15 or 20 mg/kg, or at a 1200-mg flat dose, Q3W	PD-L1 inhibitor	116	ORR, 10% (12.7% in PD-L1+) Median PFS, 1.4 months by RECIST Median PFS, 1.9 months by irRECIST Median OS, 8.9 months
IMpassion130 NCT02425891	Schmid *et al.*, 2018*[40] Emens *et al.*, 2019*[41]	High	Untreated metastatic TNBC unselected for PD-L1	Combination of immunotherapy and chemotherapy	Nab-paclitaxel ± atezolizumab 840 mg Q2W	PD-L1 inhibitor + Chemotherapy	902 (451 treated with atezolizumab)	ORR, 56% (58.9% in PD-L1+) Median PFS, 7.2 months Median PFS, 7.5 months (PD-L1+) Median OS, 21.3 months Median OS, 25 months (PD-L1+)

*Studies from the same group of researchers at different stages of study. irRECIST immune-related Response Evaluation Criteria In Solid Tumors; RECIST Response Evaluation Criteria In Solid Tumors; PD-L1 programmed death-ligand 1; TNBC triple-negative breast cancer; PFS progression-free survival; ORR objective response rate; OS overall survival; Q2W every 2 weeks; Q3W every 3 weeks.

sequentially in the population with the intention to treat and in the positive PD-L1 subgroup. A total of 902 patients were included (451 patients in each group), including 369 patients (40%) with a PD-L1 positive tumor. Approximately 25% of patients had liver metastases and 63% of patients received prior adjuvant/neoadjuvant treatment, including 51% of patients with prior exposure to taxanes. With an average follow-up of 12.9 months, atezolizumab increased marginally, but significantly, the PFS in the general population [median 7.2 months *vs* 5.5 months, HR=0.80, 95% CI (0.69-0.92), P=0.002]. However, in the subgroup of patients with positive PD-L1, the increase in PFS was more substantial and clinically relevant [median of 7.5 months *vs* 5 months, HR=0.62, 95% CI (0.49-0, 78), P<0.001]. By the time of the first interim analysis of OS (median follow-up 12 months), with less than 50% of survival events, OS was not significantly different between atezolizumab and placebo in the general population [median 23 months *vs* 17, 6 months, HR=0.84, 95% CI (0.69-1.02), P=0.08].[40,41]

However, in the subset of patients with positive PD-L1, a large and clinically significant numerical improvement in the OS was observed in the atezolizumab group [median 25 months *vs* 15.5 months, HR=0.62, 95% CI (0.45-0.86)]. It is important to note that according to the protocol, statistical significance could not be tested in this subgroup, as the improvement in OS was not confirmed in the entire population at the time. In a recently reported update (second intermediate analysis after an average follow-up of 18 months), the median OS was not yet significantly different between each group (21 months *vs* 18.7 months, stratified

Table 3. Adverse effects.

Adverse event description N(%)	Nanda *et al.*, 2016[31]	Loi *et al.*, 2017[28] Adams *et al.*, 2019[29]	Emens *et al.*, 2019[39]	Schmid *et al.*, 2018*[40] Emens *et al.*, 2019*[41]
Arthralgia	6 (18.8)	10 (5.9)	4 (3)	
Alopecia	--	--	--	255 (56.4)
ALT increased	2 (6.3)	--	5 (4)	--
AST increased	2 (6.3)	--	5 (4)	--
Anemia	1 (3.1)	--	5 (4)	--
Aseptic meningitis	1 (3.1)	--	--	--
Asthenia	--	11 (6.5)	11 (10)	--
Blood fibrinogen decreased	1 (3.1)	--	--	--
Colitis	--	2 (1.2)	--	--
Cough	--	--	--	112 (24.8)
Diarrhea	4 (12.5)	12 (7.1)	12 (10)	--
Dizziness	--	--	3 (3)	--
Decreased appetite	--	13 (7.6)	8 (7)	--
Influenza-like illness	--	--	9 (8)	--
Disseminated intravascular coagulation	1 (3.1)	--	--	--
Erythema	2 (6.3)	--	--	--
Fatigue	6 (18.8)	35 (20.6)	15 (13)	--
Headache	3 (9.4)	--	6 (5)	--
Hypothyroidism	--	20 (11.8)	5 (4)	62 (13.7)
Hyperthyroidism	--	9 (5.3)	--	--
Hyperhidrosis	--	--	4 (3)	--
Hyponatremia	--	--	3 (3)	--
Infusion-related reaction	--	3 (1.8)	--	--
Lymphopenia	1 (3.1)	--	--	--
Myalgia	6 (18.8)	--	4 (3)	--
Myocarditis	--	1 (0.6)	--	--
Nausea	5 (15.6)	19 (11.2)	13 (11)	208 (46.0)
Neutropenia	--	--	3 (3)	94 (20.8)
Pain	--	--	3 (3)	--
Peripheral neuropathy	--	--	--	98 (21.7)
Pruritus	2 (6.3)	11 (6.5)	11 (10)	--
Pyrexia	1 (3.1)	--	19 (16)	--
Pneumonitis	--	7 (4.1)	--	--
Rash	--	--	11 (10)	--
Type 1 diabetes mellitus	--	1 (0.6)	--	--
Vomiting	--	--	8 (7)	--

*Studies from the same group of researchers at different stages of study. (--) Information not available in the published article. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

HR=0.86, P=0.07) across the population, and the numerical difference in OS in the positive PD-L1 subset tended to decrease (median OS 25 months *vs* 18 months, HR=0.71, no formal P-value by protocol design). Other efficacy variables were also favorable to atezolizumab: ORR increased significantly (from 45.9 to 56%, P=0.002 and from 42.8 to 56.9%, in the general population and in patients positive for with PD-L1, respectively), including an increase in complete responses (from 1.6 to 7.1% and from 1.1% to 10.3%, in the general and positive PD-L1 populations, respectively) and the duration of responses was increased (from 5.6 to 7.4 months and from 5.5 to 8.5 months, in the general population and in the positive PD-L1 subgroup, respectively).[40,41]

Adverse effects such as nausea, cough, neutropenia, pyrexia and hypothyroidism were more frequent in the atezolizumab intervention group. Potentially immune-related events that reached a grade 3-4 were observed in 7.5% of patients treated with atezolizumab, against 4.3% in the placebo group, as seen in Table 3.[40]

Conclusions

Through the review of immunotherapy with anti-PD-1/PD-L1 agents in the treatment of TNBC, it is clear that a new therapeutic option is emerging as a new treatment in breast cancer. Anti-PD-1/PD-L1 agents as monotherapy have shown encouraging results in the metastatic setting, especially when administered earlier in the course of the disease, although combination strategies appear to increase the responses. It is clear that for patients with advanced TNBC with PD-L1+, CD8+ or TIL+ markers, the ideal treatment would include initial atezolizumab and nab-paclitaxel.

The use of an anti-PD-1 or anti-PD-L1 agent, or the use of both of them seems to be relevant for the survival rate even in patients that did not receive immunotherapy as the first-line therapy. New studies with patients without a positive immune infiltrate must be conducted, in order to verify the efficiency in this setting, as well as new clinical trials that associate immunotherapy with other agents in the first-line therapy. New predictive biomarkers also need to be developed. To date, IHC is the only FDA-approved test for measuring PD-L1 expression.

There is no doubt that the anti-PD-1/PD-L1 agents will be part of the therapeutic arsenal of breast cancer in a near future. It is hoped that in the future new studies that start to use immunotherapy earlier and as a first choice may bring more positive results to this new therapeutic modality.

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